IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	Lyons et al.	PATENT
Serial No.:	10/732,862	Attorney Docket LOR-136.0 (9720/88881)
Filed:	December 10, 2003	
For:	STABILIZED IMMUNOGENIC HBc CHIMER PARTICLES	Group Art No. 1648
Examiner:	Bo Peng	
Confirmation No. 9117		

APPELLANTS' REPLY BRIEF ON APPEAL

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Reply Brief is in response to the Examiner's Answer, mailed November 9, 2010.

Applicable fees accompany the filing of this brief. Should there be any deficiency in fees in connection with this Appeal, the Commissioner is respectfully requested and is hereby authorized to charge any such deficiency in fees to Deposit Account No. 23-0920.

STATUS OF CLAIMS

Claims 1-47 are pending and have been twice rejected. The rejections of claims 1-47 are being appealed. A copy of the pending claims appears in the Claims Appendix.

In the Examiner's Answer mailed November 11, 2010, the Fifth ground of rejection concerning claims 1-46 on the ground of nonstatutory obviousness-type double patenting over claims of application serial number 10/805,913 and claims of application serial number 10/806,006 was found to be moot because those applications were abandoned.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1) Whether Claims 1-47 are unpatentable under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

2) Whether Claims 1-47 are unpatentable under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

3) Whether Claims 1-6, 8-14, 16-28, 30-42 and 46 are unpatentable under 35 U.S.C. §103 as being obvious over Pumpens et al. (*Intervirology*, 1995, vol. 33, pp. 63-74) in view of Zlotnick et al. (*PNAS*, 1997, vol. 94, pp. 9556-9561) and Zheng (*J. Biol. Chem.*, 1992, vol. 267, pp. 9422-9429).

4) Whether Claims 1-47 are unpatentable under 35 U.S.C. §103 as being obvious over Page et al. (WO 01/98333) and Birkett (U.S. Patent Number 6,231,864) in view of Zheng (*J. Biol. Chem.*, 1992, vol. 267, pp. 9422-9429).

5) Whether Claims 1-46 are unpatentable under obviousness-type double patenting, as being obvious over (1) claims 1-78 of 09/930,915; (2) claims 1-53 of 10/787,734; (3) (withdrawn by Examiner); (4) claims 79-115 of 10/806,006; (5) claims 47-85 of 11/508,655; (6) (withdrawn by Examiner).

6) Whether Claims 1-6, 8-28, and 30-46 are unpatentable under obviousness-type double patenting, as being obvious over

claims 1-19 of U.S. Patent Number 6,231,864 in view of Page et al. (WO 01/98333) and Zheng (*J. Biol. Chem.*, 1992, vol. 267, pp. 9422-9429.

ARGUMENT

1) Claims 1-47 Comply with the Written Description Requirement under 35 U.S.C. §112

In contrast to allegations asserted in the Examiner's Answer, mailed November 9, 2010, Claims 1-47 do satisfy the written description requirement. The Office Action of March 3, 2009 rejected claims 1-47 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement and the Answer reiterated the rejection for convenience.

The Answer stated that the claims are directed to HBc chimers up to about 600 amino acids that can self assemble into particles, wherein the HBc contains 5% substitution (mutations) corresponding to the wild-type HBc of SEQ ID NO:1, and one or both cysteines at positions 48 and 107 is replaced by another residue, wherein the HBc contains heterologous epitopes at the N-terminus, immunodominant loop and/or the C-terminus. The Answer stated that the scope of the claims encompasses a large number of HBc variants, which contain 5% substitutions corresponding to SEQ ID NO:1. The Answer then stated that the claims require such HBc chimers can form particles and have enhanced stability, citing claim 25(iii).

The Answer alleged that the data of Table 13 showed that it is uncertain if HBc chimers containing a 5% substitution frequency of SEQ ID NO:1 can form viral-like particles as can wild-type HBc or whether the resultant HBc particles have enhanced stability as claimed. The Answer stated that 17 of the 24 modified chimers tested lost their ability to form particles. The Answer stated that chimer 1569 of Table 13 yielded 11.2 mg of particles, while its counterpart chimer 1789 yielded 0.4 mg of particles and that these chimers had the same epitope. The Answer alleged that the only difference between these chimers was the cysteine substitutions. The Answer alleged that the instability of the chimer 1789 does not appear to be due to the epitope inserts as argued in the Appeal Brief.

It is submitted that only 4 molecules of the 24 shown in Table 13 have cysteine 48 and/or 107 substitutions and an epitope insert per the recited claim limitations. (See, chimer nos. 1794, 1775, 1780, and 1789.) Of those 4 molecules that contain all the claim limitations, only 2 of the corresponding non-cysteine-substituted molecules formed particles. (See, chimer nos. 1546 and 1569.) Therefore, it was reasonably expected that only 2 of the molecules containing cysteine 48 and/or 107 substitutions could form particles, which is what was reported in the specification. (See, chimer nos. 1775 and

1789.) Therefore, the Answer's allegation that 17 of the 24 HBc chimers of Table 13 lost their ability to form particles is erroneous and misleading because only two of those HBc chimers are relevant and contain all the limitations of the claims. Two additional HBc chimers are not relevant and do not contain all of the claim limitations because the wild type chimers did not form particles, and therefore a cysteine-substituted chimer could not be "more stable by size exclusion chromatography after storage at 37° C in a 20 mM sodium phosphate buffer at pH 6.8 for a time period of one month than are particles formed from otherwise identical HBc chimer molecules that contain both cysteine residues at positions 48 and 107" because there was nothing to it could be compared. With regard to Table 13, it would be truthful and more accurate to state that every HBc chimer featuring the claim limitations formed particles when the corresponding substantially identical HBc chimer without cysteine substitutions formed particles.

The present claims recite that the modified HBc chimers are more stable than otherwise identical HBc chimers that contain cysteines at positions 48 and 107. One of skill would reasonably understand this to mean that if the wild-type HBc chimers that contain cysteines at positions 48 and 107 do

not form particles, neither will the HBc chimers that do not contain cysteines at positions 48 and 107.

It is submitted that Table 13 demonstrates that chimers whose wild-type counterpart formed particles, also formed particles (chimer nos. 1775 and 1789) when the cysteines were substituted. The ASP-1 epitope cysteine 48- and/or 107-substituted form (chimer no. 1775) formed more particles and was more stable than the cysteine 48- and/or 107-containing form (chimer no. 1546). Thus, the written description requirement is complied with.

It was previously noted that the β-amyloid epitope, which failed to assemble into a stable particle in cysteine 48- and/or 107-containing form (chimer no. 1510), also failed to assemble in cysteine 48- and/or 107-substituted form (chimer no. 1794). The anthrax epitope failed to assemble into a stable particle in the non-cysteine-substituted form (chimer no. 1629) and also failed to assemble in the engineered, cysteine-substituted form (chimer no. 1780). The influenza epitope in the non-cysteine-substituted form (chimer no. 1569) formed particles as did the engineered form (chimer no. 1789).

However, there was a purification problem, as explained in the specification, with the influenza engineered particle as the engineered form seemed to stick to the HYPATITE™

column and recovery was therefore lower than the non-engineered form. This problem was verified because the data showed that the particle yield for Cys-replaced chimer no. 1789 was high (77 AU), but that the purified product yield was low (0.4 mg), where, theoretically, it should also have been high if no substantial purification problems developed, as did here with the protein sticking to the column.

In short, Table 13 provides evidence that C48S/C107S-substituted, epitope-added chimer forms have similar capabilities in forming particles as do their non-C48S/C107S-substituted counterparts. This is confirmed by the data in Table 12, which show that C48S/C107S molecules having C-terminal cysteines without epitope inserts also successfully assembled into particles. Therefore, because there is evidence in the specification that the claimed molecules, which contain both epitope inserts, C48/C107 substitutions and one or more terminal cysteines, successfully formed particles that were more stable than the wild-type, this evidence reasonably conveys to one skilled in the art that the inventors had possession of the claimed invention at the time the application was filed.

Moreover, the specification stated: "[o]f particular note was the fact that the C-terminally stabilized C48S/C107S chimer appeared to be entirely disulfide bonded at day zero,

whereas its C48/C107 counterpart was not and did not reach the same level of cross-linking achieved by C48S/C107S chimers during the period of study." (See, page 192, first paragraph) These two facts undeniably show that there is a positive correlation between substitution of cys48 and cys107 and enhanced stability of the chimer. Therefore, the written description requirement has been complied with.

To reiterate what has been said before, the Federal Circuit's predecessor held that it is not necessary that every permutation within an operable invention be effective in order for an inventor to obtain a generic claim. *In re Angstadt and Griffin*, 190 USPQ 214 (CCPA 1976). Nevertheless, no pair of otherwise identical constructs has been noted in any action in which a construct that formed particles when both cysteines were present was not more stable with those cysteines replaced as recited in the claims. Therefore, it is respectfully requested that this rejection be withdrawn.

Next, the Answer stated that the art indicates that the result of peptide modification is, in general, unpredictable, citing Metzger et al. This is not what Metzger indicated in her article, but quite the opposite. Metzger stated that proline 138 was a highly conserved residue among species and that therefore <u>it was expected</u> that it was

functionally and structurally important and that substituting it would cause problems (see Figure 3 of Metzger). Therefore, Metzger taught that it was possible to predict which amino acid could be mutated and which could not, based on conserved amino acids.

Still further, the disclosure at page 5 of the application cites and exemplifies the Metzger teachings about the importance of Pro 138 so that a skilled worker to whom the disclosure is directed would know the importance of keeping that residue in the sequence rather than altering or omitting it.

The Court in *Angstadt,* mentioned above, went on to discuss the disclosure that there taught how to make and how to use a claimed catalyst. It continued that if a skilled worker wanted to make a catalyst other than those specifically disclosed in the 40 examples, that worker could simply follow the disclosure and make a desired catalyst compound. It is not understood why, about 30 years after *Angstadt* was decided, a worker of ordinary skill would be less sophisticated and ignore the teachings provided.

The claims are therefore enabled and allowable, and this rejection should be withdrawn.

2) Claims 1-47 Comply with the Enablement Requirement

Claims 1-47 do comply with the scope of enablement requirement under 35 U.S.C. §112, first paragraph. The Answer alleged that the specification, while being enabling for a HBc chimer of SEQ ID NO:1, does not reasonably provide enablement for a HBc chimer containing up to about 5% substituted amino acid residues. The Answer alleged that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

In response to the allegations above, it is submitted that there is ample evidence in the specification for chimer molecules having no more than about 5% substitution in the HBc sequence that form particles. Figures 1A and 1B of the specification show the HBc amino acid sequences of three human HBc subtypes and two mammalian species, woodchuck and ground squirrel. It can be seen that the amino acid sequences are similar though not identical, each having numerous amino acid substitutions (about 200 total) as compared to the most preferred subtype HBc *ayw* SEQ ID NO:247. Yet, these sequences all form particles. Neither the Actions nor the Answer has provided any basis to believe that a skilled worker could not

use any of those different sequences and not arrive at a particle.

Also, the specification recites that substitutions, other than in the immunodominant loop or at the termini, are preferably in the non-helical portions, between residues 1-15 and 24-50, approximately, to help assure particle formation, citing Koschel et al. (See, page 75 of the specification at least.) This disclosure provides additional guidance besides the recited LASERGENE software in predicting what substitutions will be tolerated for folding.

The specification also cited Kratz et al., who showed that amino acids 79 and 80 could be replaced and the molecule still formed particles. (See page 7 of the specification.)

The specification related that U.S. Patent No. 5,990,085 described two particle-forming fusion proteins formed from bovine inhibin peptide fused between residues 78 and 79 and after residue 144. The specification also related that U.S. Patent No. 6,231,864 described strategically modified HBc core protein linked to a hapten, which formed particles and contained an insert of about 1-40 residues. The specification also teaches that recently published WO 01/27281 taught that the presence of several residues of the native HBc sequence upstream of the C-terminal cysteine was not required to form particles.

Similarly, recently published WO 01/98333 taught the deletion of particle-forming mutants as did WO 02/13765 and WO 02/14478. Also, pages 10-11 of the specification list several other teachings related to particle formation and which residues of HBc that may be involved. The above teachings are sufficient and can be used by one skilled in the art to reproducibly prepare stable chimer particles.

Another article discussed in the specification was that of Zlotnick et al., who showed that molecules having amino acid substitutions at positions 48, 61, 107 and 150 formed particles. (See, Zlotnick, page 4, first paragraph).

As further evidence of particles having amino acid substitutions, as noted before, the specification describes the work of Metzger et al., who showed that substitutions of the 4 prolines in the sequence found in the region of sequence 129-144 formed particles, except for the proline 138 position. Metzger stated that this lack of substitution at this position was predictable and not unexpected because proline 138 plays a crucial role in the assembly of particles. (See, Metzger, page 589, column 2.) The skilled worker would therefore know to include the proline at position 138 to help assure particle formation.

Thus, if he/she did not already know how to make and use HBc chimer particles, a skilled worker would have been able to make and use such particles from the inventors' teachings in the specification and the teachings of the articles cited throughout the specification, which teachings are directed to workers of ordinary skill in the art. Such particles would, however, not have been within the claimed subject matter, but would rather be the standards against which particles of a claimed chimer protein molecule would be tested for enhanced stability.

The primary bases of the various Actions and the Answer for the assertion that the full breadth of the subject matter claimed is not enabled are the misapprehensions of what the skilled worker would take away from reading the specification, including the Metzger paper and its admonition about the effects of an absence of a proline at position 138, and that no specific examples of chimer proteins with conservative substitutions in the HBc sequence portion have been shown.

The Court in *In re Strahilevitz,* 212 USPQ 561; 668 F.2d 1229 (Fed. Cir. 1982) held that where there were there were sufficient literature citations to establish both the level of ordinary skill in the art and the fact that the techniques

necessary to practice the invention were known in the art, enablement could be found. It is submitted that far more was present here than in *Strahilevitz*. Not only is the specification rife with literature citations that here also set the level of skill in the art as well as show that a skilled worker could make and use the claimed subject matter, but the specification contains several actual examples of subject matter that falls within the claims. *Strahilevitz* had no such examples.

Having been dealt with the misunderstanding of what the Metzger teaching recited in the specification would teach a skilled worker, the only basis remaining for the rejection is an amorphous and unsupported assertion of a lack of enablement. The Court in *In re Marzocchi & Horton*, 439 F.2d 220, 223; 169 USPQ 367, 369-370 (CCPA 1971) ruled upon the propriety of a similar rejection in a chemical application. The Court held that the

> only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion [of efficacy]. [169 USPQ at 369; emphasis in the original.]

The Court went on to hold:

> it is encumbent upon the Patent Office, whenever a rejection on this basis [doubt as to enablement] is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate statement. (Emphasis in the original.)

The *Marzocchi* requirement of an explanation of "why" truth or accuracy of the disclosure is doubted has not been complied with in that neither the Actions nor the Answer have provided "acceptable evidence or reasoning which is inconsistent with the contested statement". Metzger teaches what should be done to get particles. The specification also teaches how to make and use numerous sequences that form particles and shows that adding the C-terminal cysteine aids stability of those particles once formed.

Thus, the claims are enabled. It is therefore respectfully requested that this rejection be withdrawn.

3) Obviousness Has Not Been Established as to Claims 1-6, 8-14, 16-28, 30-42 and 46

Claims 1-6, 8-14, 16-28, 30-42 and 46 are not obvious under 35 U.S.C. §103. These claims were rejected as allegedly

prima facie obvious over Pumpens et al. (1995, Intervirology, vol. 33, pp. 63-74), in view of Zlotnick (1997, PNAS, vol. 94, pp. 9556-9561), and Zheng (1992, J. Biol. Chem., vol. 267, pp. 9422-9429).

The Answer stated, in regard to Pumpens, that this art did not teach away but provided motivation to solve the problems of HBc as a vaccine carrier. (See, page 22 of Answer.) The Answer also stated that a prior art reference may be considered to teach away when a person of ordinary skill would be discouraged from following the path set out in the reference or led in a divergent direction, citing *In re Gurley.* Considering that which Pumpens teaches and that which is claimed here, they are not the same, with Pumpens providing no basis to be pursued as is detailed below.

Pumpens teaches that C-terminally truncated HBcΔ is less stable than the corresponding full-length particle. (See, page 67, first paragraph of Pumpens.) However, a claimed chimer here is C-terminally truncated. Thus, Pumpens teaches away from the claimed invention. Pumpens also failed to teach replacement of the cysteine residues at positions 48 and 107, let alone the effect of that replacement.

The Answer next disputed that Zlotnick also teaches away from the present invention. We disagree. As repeatedly

pointed out, the very first sentence of paragraph 1 states: "[p]urified Cp*149 and Cp*150 assemble into capsids under the same conditions as other Cp constructs with or without DTT." This means that there is <u>no benefit</u> as to having a C-termminal cysteine. Cp*149 has no cysteines, yet it still assembled into stable particles. Cp*150 in DTT, the reduced form, still assembled into stable particles.

The second sentence of that paragraph states: "[t]hese capsids were indistinguishable by negative staining electron microscopy and sedimentation on sucrose gradients (data not shown)." One of skill would understand that this sentence also means that there is no benefit as to having a C-terminal cysteine. Zlotnick plainly states that no advantage was seen by the results of two different analytical methods. Again, the Answer has chosen to ignore this evidence of Zlotnick.

The first paragraph of Zlotnick's MATERIALS AND METHODS section on page 9557, Figure 2 is discussed under the bold faced heading "Preparation and Biochemical Studies of <u>Gold-Labeled</u> Cp*150" (emphasis added). The biochemical studies of Figure 2, whose results were quoted in the Answer on page 26, section 56, were the gel and chromatogram of <u>a C-terminal gold-labeled 150-mer HBc particle and a 149-mer HBc particle without</u>

<u>a C-terminal cysteine or gold label</u>, neither of which particles are claimed here.

The Answer stated that the Appellants' arguments relating to teaching away should not be found persuasive because Zlotnick's teaching that HBc labeled with a gold cluster is unimpaired in its ability to form an HBc particle does not mean that the C-terminal cysteine is not important for capsid formation or stabilization. We respectfully disagree. If there is no C-terminal cysteine present yet the particle still formed a capsid, this unambiguously means that the cysteine there is not important to particle formation. Zlotnick clearly uses the C-terminal cysteine to bond the protein to the gold particles.

The Answer emphasized the sentence: "[t]hese bonds stabilize the quarternary structure of the capsid, as attested by the observation that oxidized Cp*150 capsids-unlike CP*149 capsids, or reduced Cp*150 capsids-are resistant to dissociation by 3.5 M urea (Fig. 2b)." However, Figure 2b refers to C-terminal gold-cluster labeled capsids.

Zlotnick specifically stated in the second paragraph of page 9557:

> [i]n a typical reaction, 12 nmols of reduced Cp*150 were mixed with 6 nmols of monomaleimidyl-undecagold (Au11) (30)(Nanoprobes, Stony Brook, NY) to a final

> volume of 300 μl and incubated for 24 hr at 4°C. The reactants were separated on a Sephacryl S-200 HR column (1.4 cm diameter x 6 cm), eluted in 0.5 ml fractions with 100mM sodium phosphate, pH 6.9. Capsids eluted in the void volume at 3-4 ml, free dimers at 5-6 ml, and free Au11 at about 8 ml (Fig. 2).

If one of skill in the art examined Figure 2, a large peak would be seen at about fractions 8-10, which correspond to the gold-labeled capsid, reported to elute at about 4-5 ml. These gold-labeled capsids elute slightly earlier than the Cp*149 capsids and Cp*150 in DTT capsids, which makes sense because both would be a little smaller as neither has attached gold clusters. The next peaks are the dimmers.

As described above, Figure 2b refers to <u>gold-cluster-labeled capsids</u>, which the present claims do not recite. The Answer stated that it is common practice in the art of biology to study the function of a compound by using the labeled compound. (See, page 27 of Answer.) However, one of skill would understand that a labeled compound is not identical in physical structure or chemical properties to an unlabeled compound. There can be significant differences, especially with a gold-cluster label which is large. In fact, Zlotnick recognizes this in his discussion of the behavior of gold-labeled particles on page 9560.

The Answer next refers to Zheng, citing that Abstract as evidence that Zheng teaches that cys61 and cys183 are required for forming interchain disulfide bonds. The Abstract does not teach this, but instead relates:

> Each of the cysteines of HBcAg has been eliminated, either singly or in combination, by a two-step mutagenesis procedure. All of the proteins were shown to have very similar physical and immunochemical properties. All assemble into essentially identical core particle structures. Therefore disulfide bonds are not essential for core particle formation. (see page 9422, line8, emphasis added)

Therefore, it is irrelevant whether cys48, cys61 and cys107 form disulfide bonds or not. They are not essential for HB core particle formation.

All in all, the Answer has failed to show that the presently claimed invention is obvious in light of the combined teachings of Pumpens, Zlotnick and Zheng. As a consequence, the present invention is not *prima facie* obvious. This basis for rejection should be withdrawn.

4) A Prima Facie Case of Obviousness Has Not Been Established for Claims 1-6, 8-14, 16-28, 30-42, and 46

Claims 1-6, 8-14, 16-28, 30-42, and 46 are not obvious under 35 USC §103 over the disclosures of Page (WO 01/98333) and Birkett (U.S. Patent No. 6,231,864) in view of Zheng (J. Biol.

Chem., 1992, vol. 267, pp.9422-9429). The Answer stated that one of ordinary skill would have been motivated to modify HBcΔ by incorporating the features of cysteine 48 and 107 substitution and cysteine 61 retention as taught by Zheng and to include an epitope at the N-terminus of the molecule as taught by Page. The Answer stated that there would have been a reasonable expectation of success given the knowledge that HBc chimeras containing a chemically-reactive linker residue for a conjugated hapten at their immunodominant loop have been successfully made by Birkett. The Answer then alleged that the invention was *prima facie* obvious. We respectfully disagree.

The Answer stated that Zheng teaches that cys61 and cys183 are required for forming interchain disulfide bonds with another monomer, resulting in HBc dimers. However, as shown in the discussion above, which is reiterated here by reference, Zheng clearly does not teach that cys61 and cys183 are required for core particle formation as alleged.

Moreover, Page does not teach the substitution of cysteines 48 and 107 and the retention of cysteine 61. Furthermore, Birkett does not teach does not teach the substitution of cysteines 48 and 107 and the retention of cysteine 61. Therefore, this combination fails to teach or make obvious the present invention.

As for the teaching that a C-terminal cysteine is stabilizing, neither Zheng, Birkett nor Page teaches this. Zheng has been addressed above, Birkett only teaches full-length HBc molecules and the citation to Page provided in the Answer only refers to the native structure of HBc. Thus, this combination of art does not teach the recited limitations or make the claims obvious. It is respectfully requested that this rejection be withdrawn.

5) Claims 1-46 Are Not Obvious under <u>Obviousness-Type Double Patenting</u>

Claims 1-46 were provisionally rejected under obviousness-type double patenting over claims 1-78 of No. 09/930,915; claims 1-53 of 10/787,734 (now U.S. Patent No. 7,361,352); and claims 47-85 of 11/508,655. The other bases were withdrawn by the Examiner. A terminal disclaimer is believed premature at this time. These claims are allowable.

6) Claims 1-6, 8-28, and 30-46 Are Not Obvious under <u>Obviousness-Type Double Patenting</u>

Claims 1-6, 8-28, and 30-46 were rejected for obviousness-type double patenting over claims 1-19 of U.S. Patent No. 6,231,864 in view of Page et al. (WO 01/98333) and Zheng (1992, J. Biol. Chem., vol. 267 (13): pp. 9422-9429). The

combined teachings do not make the invention obvious as none at all teaches or suggests the replacement of Cys48 and/or Cys107, nor the resulting enhanced stability, as argued in previous sections, which are incorporated herein by reference. The claims are patentably distinct and are allowable.

CLAIMS APPENDIX

1. (rejected) A recombinant chimer hepatitis B core (HBc) protein molecule up to about 600 amino acid residues in length that

(a) contains an HBc sequence of at least about 125 of the N-terminal 183 amino acid residues of the HBc molecule that includes the HBc sequence of residue positions 4 through about 75 and about 85 through about 140 in which one or both cysteine residues at positions 48 and 107 is replaced by another residue, and in which the cysteine at residue position 61 is present;

(b) contains a peptide-bonded heterologous amino acid residue sequence at one or more of the N-terminus, in the HBc immunodominant loop between residue positions about 76 through about 85 or the C-terminus of the chimer, and wherein

[(i)] (1) zero to all residues in a sequence in said HBc immunodominant loop are present or replaced and said heterologous amino acid residue sequence comprises one to about 245 amino acid residues that constitute an immunogen or a sequence of 1 to about 40 residues that constitutes an anti-antigen or a chemically-reactive linker residue for a conjugated hapten; or

[(ii)] (2) the sequence of HBc at positions 76 through 85 is present and free from deletions and heterologous residues; or

[(iii)] (3) one or more of residues 76 through 85 is absent or replaced;

(c) contains one or both of

[(i)] (1) one to three cysteine residues at an amino acid position of the chimer molecule corresponding to amino acid position -20 to about +1 from the N-terminus of the HBc sequence of SEQ ID NO:1 [N-terminal cysteine residue(s)] in a sequence other than that of the HBc precore sequence and

[(ii)] (2) one to three cysteine residues toward the C-terminus of the molecule from the C-terminal residue of the HBc sequence and within about 30 residues from the C-terminus of the chimer molecule [C-terminal cysteine residue(s)]; said chimer molecule

(i) having an amino acid sequence in which up to about 5 percent of the amino acids are substituted in the HBc chimer sequence corresponding to SEQ ID NO:1, and

(ii) self-assembling into particles that exhibit a ratio of absorbance at 280 nm to 260 nm of about 0.9 to about 1.7 after expression.

2. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 wherein the N-terminal sequence includes a heterologous sequence containing up to about 75 amino acid residues peptide-bonded to one of HBc residues 2-4 that includes an immunogenic epitope.

3. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 wherein the sequence of HBc at position about 76 through about 85 is present and free from deletions and heterologous residues.

4. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 wherein zero to all residues in a sequence of HBc positions 76 through 85 are present and peptide-bonded to one to about 245 amino acid residues that are heterologous to HBc and constitute a heterologous epitope.

5. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 wherein one or more of residues 76 through 85 is absent or replaced.

6. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 wherein the C-terminal sequence contains up to about 100 amino acid residues that include an immunogenic epitope in a sequence heterologous to HBc and bonded to said C-terminal residue of the HBc sequence.

7. (rejected) The recombinant chimer hepatitis B core (HBc) protein molecule according to claim 1 wherein the HBc residue at each of positions 76 and 82 is replaced by a cysteine residue.

8. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 that contains an HBc sequence of at least about 125 of the N-terminal 163 amino acid residues of the HBc molecule.

9. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 that is up to about 380 amino acid residues in length.

10. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 1 that contains at least about 135 of the N-terminal 163 amino acid residues of HBc.

11. (rejected) A recombinant chimer hepatitis B core (HBc) protein molecule up to about 380 amino acid residues in length that

(a) contains an HBc sequence of at least about 125 of the N-terminal 163 amino acid residues of the HBc molecule that includes the HBc sequence of residue positions 4 through about 75 and about 85 through about 140 in which one or both cysteine residues at positions 48 and 107 is replaced by another residue, and in which the cysteine at residue position 61 is present;

(b) includes one or more of the following:

(i) a peptide-bonded heterologous sequence of up to about 75 residues at one or more of the N-terminus, in the HBc immunodominant loop and at the C-terminus of the chimer wherein that C-terminal sequence is other than that of HBc from position 163 through the native HBc C-terminus,

(ii) zero to all of the residues of the sequence of position about 76 through about 85 are present or replaced , wherein

(iia) said heterologous sequence of up to about 75 amino acid residues is peptide-bonded to the sequence between about positions 76 through about 85, or

(iib) a sequence of one to about 40 amino acid residues that constitute an anti-antigen is peptide-bonded to the sequence between about positions 76 through about 85, or

(iic) a chemically-reactive linker residue for a conjugated hapten is peptide-bonded to the sequence between about positions 76 through about 85, or

(iid) the sequence of HBc at position about 76 through about 85 is present and free from deletions and heterologous residues, or

(iie) one or more of residues about 76 through about 85 is absent or replaced;

(c) contains one to three cysteine residues present

(i) at an amino acid position of the chimer molecule corresponding to amino acid position -20 to about +1 from the N-terminus of the HBc sequence of SEQ ID NO:1 [N-terminal cysteine residue(s)] in a sequence other than that of the HBc precore sequence, or

(ii) toward the C-terminus of the molecule from the C-terminal residue of the HBc sequence and within about 30 residues from the C-terminus of the chimer molecule [C-terminal cysteine residue(s)], or at both locations (i) and (ii);

(d) has an amino acid sequence in which up to about 5 percent of the amino acids are substituted in the HBc chimer sequence corresponding to SEQ ID NO:1, and

(e) self-assembles into particles after expression that upon collection, purification and dissolution, exhibit a ratio of absorbance at 280 nm to 260 nm of about 0.9 to about 1.7.

12. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 11 that contains one to three C-terminal cysteine residue(s).

13. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 11 that contains at least about 135 of the N-terminal 163 amino acid residues of HBc.

14. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 13 that contains an HBc sequence of at least about 135 of the N-terminal 156 amino acid residues of the HBc molecule.

15. (rejected) The recombinant chimer hepatitis B core (HBc) protein molecule according to claim 11 wherein the residue

of HBc at each of positions 76 and 82 is replaced by a cysteine residue.

16. (rejected) The recombinant HBc chimer protein molecule according to claim 11 wherein said peptide-bonded sequence of up to about 75 residues is present.

17. (rejected) The recombinant HBc chimer protein molecule according to claim 16 wherein said peptide-bonded sequence of up to about 75 residues is present bonded at the N-terminus of the chimer.

18. (rejected) The recombinant HBc chimer protein molecule according to claim 16 wherein said peptide-bonded sequence of up to about 75 residues is present bonded in the HBc immunodominant loop of the chimer.

19. (rejected) The recombinant HBc chimer protein molecule according to claim 16 wherein said peptide-bonded sequence of up to about 75 residues is present bonded at the C-terminus of the chimer.

20. (rejected) The recombinant HBc chimer protein molecule according to claim 16 that contains a second peptide-bonded sequence of up to about 75 residues present bonded to the N-terminus, in the HBc immunodominant loop or to the C-terminus of the chimer at a position different from that to which the first-named sequence of up to about 75 residues was bonded.

21. (rejected) The recombinant HBc chimer protein molecule according to claim 20 wherein said first-named sequence of up to about 75 residues contains a B cell epitope.

22. (rejected) The recombinant HBc chimer protein molecule according to claim 21 wherein said second-named sequence of up to about 75 residues contains a T cell epitope.

23. (rejected) The recombinant HBc chimer protein molecule according to claim 11 wherein both cysteine residues at positions 48 and 107 are replaced by another residue.

24. (rejected) The recombinant HBc chimer protein molecule according to claim 23 wherein the replacement residue for each cysteine is selected from the group consisting of glutamine, asparagine, serine, alanine, threonine and lysine.

25. (rejected) A recombinant hepatitis B virus core (HBc) protein chimer molecule that has a length of about 135 to about 365 amino acid residues and contains four peptide-linked amino acid residue sequence domains from the N-terminus that are denominated Domains I, II, III and IV, wherein

Domain I comprises about 72 to about 150 amino acid residues whose sequence includes:

[(i)] (a) at least the sequence of the residues of position 4 through position 75 of HBc,

[(ii)] (b) the substitution of another residue for the cysteine residue at position 48, while maintaining the cysteine at residue position 61,

[(iii)] (c) zero to three cysteine residues at an amino acid position of the chimer molecule corresponding to amino acid position -20 to about +1 from the N-terminus of the HBc sequence of SEQ ID NO:1 [N-terminal cysteine residue(s)] in a sequence other than that of the HBc precore sequence, and

[(iv)] (d) an optional immunogenic epitope sequence containing up to about 75 amino acid residues peptide-bonded to one of HBc residues 2-4;

Domain II comprises up to about 60 amino acid residues peptide-bonded to HBc residue 75 of Domain I in which those peptide-bonded amino acid residues comprise

(a) the sequence of 10 residues of HBc positions 76 through 85 present, but interrupted by

[(i)] (1) one to about 50 residues of a heterologous immunogen-containing sequence, or

[(ii)] (2) 1 to about 40 residues of an anti-antigen-containing sequence, or

[(iii)] (3) 1 to about 40 residues of a sequence containing a chemically-reactive linker residue for a conjugated hapten, or

(b) the sequence of HBc positions 76-85 is present with two replacement cysteine residues at HBc positions 76 and 82, and includes an interrupting sequence of

[(i)] (1) up to 50 residues of a heterologous immunogen-containing sequence, or

[(ii)] (2) 1 to about 40 residues of an anti-antigen-containing sequence; or

[(iii)] (3) 1 to about 40 residues of a sequence containing a chemically-reactive linker residue for a conjugated hapten;

Domain III comprises an HBc sequence from position 86 through position 135 peptide-bonded to residue 85 of Domain II in which another residue is substituted for the cysteine of position 107;

Domain IV comprises:

[(i)] (a) five through about thirty residues of an HBc amino acid residue sequence from position 136 through about 165 peptide-bonded to the residue of position 135 of Domain III,

[(ii)] (b) zero to three cysteine residues [C-terminal cysteine residue(s)] within about 30 residues from the C-terminus of the chimer molecule,

[(iii)] (c) zero to about 75 amino acid residues in a sequence other than that present in HBc from position 165 to the C-terminus, and the sequence of the chimer molecule from HBc position 150 through the C-terminus of the chimer molecule contains fewer than about ten arginine or lysine residues or mixtures of both residues; said chimer molecule

(i) having an amino acid residue sequence in which up to about 5 percent of the amino acid residues are substituted in the HBc sequence of the chimer corresponding to SEQ ID NO:1,

(ii) having at least one cysteine residue present from the recited zero to three cysteine residues of Domains I and IV, and

(iii) self-assembling into particles on expression by a host cell wherein the particles so formed exhibit a ratio of absorbance at 280 nm to 260 nm of about 0.9 to about 1.7 and are more stable by size exclusion chromatography after storage at 37° C in a 20 mM sodium phosphate buffer at pH 6.8 for a time period of one month than are particles formed from otherwise identical HBc chimer molecules that contain both cysteine residues at positions 48 and 107.

26. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 25 that contains one to three C-terminal cysteine residue(s).

27. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 25 that contains at least about 135 of the N-terminal 156 amino acid residues of HBc.

28. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 27 that contains an HBc

sequence of at least about 135 of the N-terminal 149 amino acid residues of the HBc molecule.

29. (rejected) The recombinant chimer hepatitis B core protein molecule according to claim 25 wherein the residue of HBc at each of positions 76 and 82 is replaced by a cysteine residue.

30. (rejected) The recombinant HBc chimer protein molecule according to claim 25 wherein a peptide-bonded sequence of up to about 75 residues is present.

31. (rejected) The recombinant HBc chimer protein molecule according to claim 30 wherein said peptide-bonded sequence of up to about 75 residues is present bonded at the N-terminus of the chimer.

32. (rejected) The recombinant HBc chimer protein molecule according to claim 30 wherein said peptide-bonded sequence of up to about 75 residues is present bonded in the HBc immunodominant loop of the chimer.

33. (rejected) The recombinant HBc chimer protein molecule according to claim 30 wherein said peptide-bonded sequence of up to about 75 residues is present bonded at the C-terminus of the chimer.

34. (rejected) The recombinant HBc chimer protein molecule according to claim 30 that contains a second peptide-bonded sequence of up to about 75 residues present bonded to the N-terminus, in the HBc immunodominant loop or to the C-terminus of the chimer at a position different from that to which the first-named sequence of up to about 75 residues was bonded.

35. (rejected) The recombinant HBc chimer protein molecule according to claim 34 wherein said first-named sequence of up to about 75 residues contains a B cell epitope.

36. (rejected) The recombinant HBc chimer protein molecule according to claim 35 wherein said B cell epitope is peptide-bonded at a position in the HBc sequence between amino acid residues 76 and 85, and at least 5 residues of the HBc sequence of positions 76 through 85 are present.

37. (rejected) The recombinant HBc chimer protein molecule according to claim 36 wherein the HBc sequence between amino acid residues 76 and 85 is present, but interrupted by said B cell epitope.

38. (rejected) The recombinant HBc chimer protein molecule according to claim 35 wherein said second-named sequence of up to about 75 residues contains a T cell epitope.

39. (rejected) The recombinant HBc chimer protein molecule according to claim 38 wherein said T cell immunogenic epitope is peptide-bonded to the C-terminal HBc amino acid residue.

40. (rejected) The recombinant HBc chimer protein molecule according to claim 39 wherein at least one of said C-terminal cysteine residue(s) is present.

41. (rejected) The recombinant HBc chimer protein molecule according to claim 25 wherein said chimer contains the uninterrupted HBc amino acid residue sequence of position 4 through at least position 140, plus a cysteine residue at the C-terminus of the HBc chimer protein molecule.

42. (rejected) The recombinant HBc chimer protein molecule according to claim 41 wherein said chimer contains the uninterrupted HBc amino acid residue sequence of position 4 through position 149.

43. (rejected) The recombinant HBc chimer protein molecule according to claim 25 wherein said chimer contains a heterologous linker residue for a conjugated epitope present in the HBc immunodominant loop.

44. (rejected) The recombinant HBc chimer protein molecule according to claim 43 wherein said heterologous linker residue for a conjugated epitope is peptide-bonded at a position in the HBc sequence between amino acid residues 76 and 85, and at least 4 residues of the HBc sequence of positions 76 through 85 are present.

45. (rejected) The recombinant HBc chimer protein molecule according to claim 44 wherein the HBc sequence between amino acid residues 76 and 85 is present, but interrupted by said heterologous linker residue for a conjugated epitope.

46. (rejected) The recombinant HBc chimer protein molecule according to claim 25 wherein the residue substituted for each cysteine at positions 48 and 107 is individually selected from the group consisting of glutamine, asparagine, serine, alanine, threonine and lysine.

47. (rejected) A recombinant chimer hepatitis B core (HBc) protein molecule up to about 600 amino acid residues in length that

(a) contains an HBc sequence of at least about 125 of the N-terminal 183 amino acid residues of the HBc molecule that includes the HBc sequence of residue positions 4 through about 75 and about 85 through about 140 in which one or both cysteine residues at positions 48 and 107 is replaced by another residue, and in which the cysteine at residue position 61 is present;

(b) contains a peptide-bonded heterologous amino acid residue sequence at one or more of the N-terminus, in the HBc immunodominant loop between residue positions about 76 through about 85, and the HBc residue at each of positions 76 and 82 is replaced by a cysteine residue, or the C-terminus of the chimer, and wherein

[(i)] (1) zero to all residues in a sequence in said HBc immunodominant loop other than at position s 76 and

82 are present or replaced and said heterologous amino acid residue sequence comprises one to about 245 amino acid residues that constitute an immunogen or a sequence of 1 to about 40 residues that constitutes an anti-antigen or a chemically-reactive linker residue for a conjugated hapten; or

[(ii)] (2) one or more of residues 76 through 85 other than the cysteines at positions 76 and 82 is absent or replaced;

(c) contains one or both of

[(i)] (1) one to three cysteine residues at an amino acid position of the chimer molecule corresponding to amino acid position -20 to about +1 from the N-terminus of the HBc sequence of SEQ ID NO:1 [N-terminal cysteine residue(s)] in a sequence other than that of the HBc precore sequence and

[(ii)] (2) one to three cysteine residues toward the C-terminus of the molecule from the C-terminal residue of the HBc sequence and within about 30 residues from the C-terminus of the chimer molecule [C-terminal cysteine residue(s)]; said chimer molecule

(i) having an amino acid sequence in which up to about 5 percent of the amino acids are substituted in the HBc chimer sequence corresponding to SEQ ID NO:1, and

(ii) self-assembling into particles that exhibit a ratio of absorbance at 280 nm to 260 nm of about 0.9 to about 1.7 after expression.

Favorable consideration of this Appeal and allowance on the captioned application are respectfully requested.

Respectfully submitted,

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